

Phosphorus Distribution in Sequentially Extracted Fractions of Biosolids, Poultry Litter, and Granulated Products

Zhongqi He,¹ Hailin Zhang,² Gurpal S. Toor,³ Zhengxia Dou,⁴ C. Wayne Honeycutt,¹ Brian E. Haggard,⁵ and Mark S. Reiter⁶

Abstract: Land application of biosolids and poultry litter can benefit crop production by providing phosphorus (P) and other nutrients and organic matter. However, the bioavailability of applied P in those waste materials is directly dependent on the presence of specific P forms. In this study, we comparatively examined the P forms in biosolids and broiler litter by sequential fractionation (i.e., H₂O, 0.5 M NaHCO₃, 0.1 M NaOH, and 1 M HCl fractions) coupled with enzymatic hydrolysis. Extractable P in broiler litter was found in the order of HCl > H₂O > NaOH > NaHCO₃. Biosolid P was found in the four fractions in the following order: HCl > NaOH > NaHCO₃ > H₂O. Correlation analysis indicated that P was coextracted with Ca, Mn, Zn, Fe, and Al, but not Mg. Whereas most P was Ca bound in broiler litter, Al, Mn, and Zn played more important roles in metal P species in biosolids. The relative abundance of P species was determined to be inorganic P > hydrolyzable organic P > nonhydrolyzable organic P in each fraction. Compared with the P levels in the samples stored in a freezer, storage of the broiler litter sample at room temperature transformed part of the phytate P in the HCl fraction to other forms, resulting in more labile P in the H₂O fraction. Granulated products of poultry litter and biosolid could be used as off-farm nutrient sources. Data in this work show that granulation transformed moderate NaHCO₃- and NaOH-extractable P bilaterally to labile water-soluble P and stable HCl-extractable P in the granulated poultry litter but did not remarkably change the distribution of H₂O-, NaHCO₃-, and NaOH-extractable P in granulated products containing both poultry litter and biosolids.

Key words: Biosolids, granulated fertilizer, organic phosphorus, phosphorus forms, poultry litter, sequential fractionation.

(*Soil Science* 2010;175: 154–161)

Land application of biosolids and poultry litter can benefit crop production by providing essential nutrients, such as phosphorus (P), and organic matter (Paudel et al., 2005; Singh et al.,

2008). However, the bioavailability of applied P in soils is directly dependent upon the lability of P in the waste materials (Alleoni et al., 2008; He et al., 2006a; 2008; Ippolito et al., 2007; Schroder et al., 2008; Toth et al., 2006). A sequential fractionation procedure has been widely used to separate P into various pools of lability. This procedure involves a stepwise extraction of P in waste materials by H₂O, followed by 0.5 M NaHCO₃, 0.1 M NaOH, and 1 M HCl (Ajiboye et al., 2004; Codling, 2006; He et al., 2008). Phosphorus in the extracts is then analyzed for inorganic and organic P forms. This procedure is based on an early fractionation scheme designed for investigating the forms and transformations of soil P under various environmental conditions (Hedley et al., 1982). Originally, labile P includes the sum of P_i and P_o from water (or resin) and NaHCO₃ fractions, whereas refractory or unavailable P includes all of the other fractions. Cross and Schlesinger (1995) proposed that biological P is all the extracted organic fractions including that in the NaOH fraction as P_o may be rapidly mineralized into P_i. Alternatively, P in the four sequentially extracted fractions are termed as *water-soluble*, *bioavailable*, *potential bioavailable* (Fe/Al-bound), and *Ca-bound* P, respectively (Su et al., 2007). However, the original scheme did not include the measurement of organic P in the HCl fraction because the researchers found that the amount of organic P in the HCl fraction of their samples was negligible (Hedley et al., 1982). Since then, most researchers have simply followed the original procedure and assumed no organic P present in the HCl fraction without conducting actual measurements (Ajiboye et al., 2004; Cross and Schlesinger, 1995). He et al. (2006c) demonstrated the presence of both P_i and P_o in sequentially extracted 1 M HCl fractions of some soil and animal manure samples. Identification of possible organic P species contained in HCl fractions of the sequential fractionation scheme would provide more accurate P characterization of the samples. For instance, based on He et al. (2006c), Vadas et al. (2007) assumed 65% rather than 100% of HCl-extractable P in animal manure to exist as inorganic P in their runoff model, thus, improving the accuracy of the modeling simulation. A recent work (Huang et al., 2008) acknowledged the possible presence of organic P in the HCl fractions of biosolids, although no measurement was performed. Therefore, it is reasonable to have some measured data to clarify the concern if any HCl-extractable organic P is present in biosolid samples or not.

Biosolids have a wide range of chemical and physical properties. For example, Schroder et al. (2008) report that the mean of total P in their biosolid samples collected from 1993 to 2005 is 38.6 g kg⁻¹, with a range from 29.8 to 59.5 g kg⁻¹. Maguire et al. (2001) investigated the relationship between biosolid treatment processes and soil P availability. These treatments included digestion and Fe, Al, and lime addition. These authors (Maguire et al., 2001) found that the trend of extractable water-soluble P, iron-oxide strip-extractable P (FeO-P), and Mehlich-1 P generally followed the pattern: (soils amended with biosolids produced without the use of Fe or Al) > (biosolids produced using Fe or Al and lime) > (biosolids produced

¹USDA-ARS, New England Plant, Soil, and Water Laboratory, Orono, ME 04469. Dr. Zhongqi He is corresponding author. E-mail: zhongqi.he@ars.usda.gov

²Dept. of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK.

³Soil and Water Quality Laboratory Gulf Coast Research and Education Center, University of Florida-Institute of Food & Agricultural Sciences, Wimauma, FL.

⁴School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

⁵Biological and Agricultural Engineering, University of Arkansas, Fayetteville, AR.

⁶Eastern Shore Agricultural Research and Ext. Center, Virginia Polytechnic Institute and State University, Painter, VA.

Received August 21, 2008.

Accepted for publication February 25, 2010.

Trade or manufacturers' names mentioned in the article are for information only and do not constitute endorsement, recommendation, or exclusion by the USDA-ARS.

Copyright © 2010 by Lippincott Williams & Wilkins

ISSN: 0038-075X

DOI: 10.1097/SS.0b013e3181dae29e

using only Fe and Al salts). Whereas both the Mehlich-3 soil test and the molar ratio of P to (Al + Fe) could predict, with reasonable accuracy, changes in soil P after the addition of biosolids to the two tested soils, the predictive accuracy of these biosolid tests tended to decrease with time (from 1 to 51 days of the incubation). The authors (Maguire et al., 2001) nonetheless concluded that testing of biosolids for availability of P, rather than total P, can be a useful tool for predicting extractable P from biosolid-amended soils.

Poultry litter granulation is one potential option for litter reduction and off-farm use (Toor et al., 2007a, 2007b, 2007c). In the granulation process, poultry litter, N fertilizer, water, and other additives reagents are mixed to form granules. The granules are then heated and dried, resulting in decreased moisture content and odor in the final granulated products, which could be used in agronomically P-deficient areas, such as in turf, lawn, and gardens. However, Toor et al. (2007a) reported that granulated litter had greater amounts of water-soluble P than raw and ground litters when measured at low solid-to-water extraction ratios (<1:100). In a follow-up study, Toor and Haggard (2009) incubated two soils with these granulated products at two application rates: P based (100 kg total P ha⁻¹) and N based (160 kg plant-available N ha⁻¹). They concluded that the contents of P and trace metals (As, Cu, and Zn) were not significantly different in the soils amended with either normal litter or granulated litter products at P-based or plant-available N-based application rates. Haggard et al. (2005) also observed that pelletizing of poultry litter, which also involves heating and drying, increased the water-soluble P in the pellets. In addition, storage time could have significant effects on manure P solubility based on the observations on the swine and dairy manure samples (Gerritse and Zudec, 1977; Baxter et al., 2003; He et al., 2003b). To our knowledge, however, no such information is available for poultry litter. Thus, the major objectives of this study were to (i) comparatively examine the P forms in biosolids and broiler litter stored frozen and at room temperature by sequential fractionation coupled with enzymatic hydrolysis and (ii) to explore the impact of granulation on P distribution in granulated poultry litter products using the sequential fractionation procedure. We further hypothesized that the high concentration of metals in biosolids could reduce the impact of granulation on water-soluble P increase in these granulated products.

MATERIALS AND METHODS

Biosolids and Broiler Litter Collection

Broiler litter (mixture of feces and bedding material) collected from a local poultry farm in Northwest Arkansas was ground to pass through a 5.8-mm mesh screen and thoroughly mixed using a New Holland 352 feed mill mixer. A poultry litter subsample was frozen within 3 days of sample collection. Another batch of poultry litter subsample was stored at room temperature for the same duration of time (15 months) as the frozen samples.

Biosolids were obtained from Stuttgart Municipal Water Works in Stuttgart, AR, which were dried using a Sludge-MASTER RK Indirect Sludge Dryer after aerobic wastewater digestion. Biosolids were heated at 343°C for 3 h and then 227°C as they moved through the dryer. They were treated to Environmental Protection Agency standards for agricultural land application.

A bench-scale granulator (12D54L Pin Mixer, Mars Mineral, Inc., Mars, PA) with vibrating screw feeders (101 and 1015 Series Volumetric Screw Feeders, Acrison, Inc., Moon-

achie, NJ) was used to make granulated mixtures of poultry litter and biosolids as shown in Table 1. Granulates were moved to a vibrating fluid bed dryer that was kept at 232°C, and then granulates were dried at 121°C (see Toor et al., 2007a, for detailed information on granulating process). Dried granulates having the size fraction of 0.85 to 4.75 mm were collected using mesh screens.

Fractionation, Dilution, and pH Adjustment of Extracts

The fractionation procedure was based on a previous report by He et al. (2006b). Briefly, each sample (0.25 g) was sequentially extracted with 25 mL of deionized water (2 h), 0.5 M NaHCO₃ (pH 8.5), 0.1 M NaOH, and 1 M HCl for 16 h each. After each extraction, the extracts were centrifuged at 14,000g for 30 min at 4°C. Then, the supernatant was passed through a 0.45-μm membrane filter. The residual precipitate was washed using 5 mL water, and the washing supernatant was discarded after centrifuging to reduce the effect of extracted P in the subsequent extraction.

For analysis, 1 mL of water extract was diluted to 10 mL by adding 2.5 mL of 400 mmol/L sodium acetate buffer (pH 5.0) and deionized water. Bicarbonate extract (2 mL) was diluted to 1:5 by slowly adding 0.512 mL of 2.5 M acetic acid and water. The pH-adjusted bicarbonate fractions were set aside for 1 to 2 h to let excessive carbonic acid (CO₂) bubble out. Hydroxide fractions were diluted to 1:2 by slowly adding 0.34 mL of 2.5 M sodium acetate and 0.38 mL of 400 mmol/L sodium acetate buffer (pH 5.0) to 5 mL extract. To prevent P compounds from precipitating during pH adjustment, 0.1 mL of 100 mM EDTA was also added to the hydroxide fraction before being brought to 10 mL with additional water. Acid fractions were diluted to 1:10 by slowly adding 8 mL of 400 mmol/L unbuffered sodium acetate and 1 mL of water to 1 mL of extracts. This way, all diluted fractions were adjusted to pH 5.0, but the concentration of sodium acetate buffer was 100 mmol/L in diluted water and

TABLE 1. Formulation Ingredients Per Metric Ton (t = 1,000 kg) of Granular Product on an as-is Basis

Product-Binder	Dry Agent				Binding Agent			
	Biosolids	DCD	Poultry	Urea	LS	W	UF	
			Litter					
kg t ⁻¹								
PLU-LS	0	0	750	250	12	27	0	
PLUDCD-LS	0	23	757	220	12	27	0	
PLUB-LS	312	0	448	240	12	27	0	
PLUBDCD-LS	318	23	456	204	12	27	0	
PLU-UF	0	0	750	250	0	27	12	
PLUDCD-UF	0	23	757	220	0	27	12	
PLUB-UF	312	0	448	240	0	27	12	
PLUBDCD-UF	318	23	456	204	0	27	12	
PLU - W	0	0	750	250	0	36	0	
PLUDCD-W	0	23	757	220	0	36	0	
PLUB-W	312	0	448	240	0	36	0	
PLUBDCD-W	318	23	456	204	0	36	0	

PLU: poultry litter + urea; PLUDCD: poultry litter + urea + DCD; PLUB: poultry litter + urea + biosolids; PLUBDCD: poultry litter + urea + biosolids + DCD.

LS: lignosulfonate; UF: urea formaldehyde resin + catalyst; W: water.

hydroxide fractions, 128 mmol/L and 320 mmol/L in diluted bicarbonate and acid fractions, respectively.

Enzymes and Enzymatic Incubation

Acid phosphatases (EC 3.1.3.2) of type IV-S from potato and type I from wheat germ and phytase (EC 3.1.3.8) from *Aspergillus ficuum* were purchased from Sigma (St Louis, MO). Lyophilized nuclease P1 (EC 3.1.30.1) from *Penicillium citrinum* was purchased from Roche Diagnostics Corporation (Indianapolis, IN). One unit (U) of enzyme activity was defined as liberation of 1.0 $\mu\text{mol/L}$ of relevant product from appropriate substrates at specific incubation conditions according to the supplier's information. The stock solutions of acid phosphatases and phytase were then dispensed into microcentrifuge vials at 1 mL per vial and stored at -20°C until use. Nuclease P1 was purchased in 1- or 5-mg per bottle, and the buffer was directly added into the bottle to obtain an activity concentration of 960 U mL^{-1} . Enzyme solutions were stored according to the manufacturer's instructions.

For enzymatic incubations, the diluted and pH-adjusted extracts were further diluted 4- to 15-fold with distilled water or sodium acetate buffer to keep a final sodium acetate buffer concentration of 100 mmol/L and pH 5.0. All enzymatic incubations were carried out at 37°C for 1 h in a refrigerated (temperature-controlled) shaker (250 r.p.m.). Each incubation mixture (1.0 mL) contained 0.8 mL of extract and 0.2 mL of enzyme-buffer working solution (0.25 U acid phosphatase type IV-S from potato, 0.25 U acid phosphatase from wheat germ, and 5 U mL^{-1} nuclease P1, separately or in combination) (He et al., 2004a). Controls omitting either the enzyme or substrates were included.

P and Metal Analysis

Orthophosphate (P_i) in the incubation mixtures was quantified by a molybdate blue method (He and Honeycutt, 2005).

Three types of enzymatically hydrolyzable organic P forms were quantified based on previous substrate specificity studies (He et al., 2004a; He et al., 2004b): (i) *simple monoester P*, which was inorganic P released by potato phosphatase alone; (ii) *phytate-like P*, which was the difference between inorganic P determined after incubation with potato phosphatase plus phytase and potato phosphatase alone; and (iii) *polynucleotide P*, which was defined as the net increase in inorganic P determined after incubation with potato phosphatase, nuclease P1, and phytase compared with inorganic P determined in step (ii). Total P, Ca, Mg, Al, Fe, Mn, and Zn in sequential fractions (no digesting was needed) and residues (after acid wet digestion) were determined with an inductively coupled plasma atomic emission spectroscopy (Plasma 400 Emission Spectrophotometer, Perkin-Elmer, Norwalk, CT). Nonhydrolyzable organic P was calculated as the difference between total P and the sum of other identified P forms as previously described.

Statistical Analysis

The data analysis package in Microsoft Excel was used for statistical analysis. Data from three laboratory replicates were used to calculate averages and SE. Single-factor analysis of variance was used to evaluate the significance of differences in P forms in the same fraction or the significance of differences in P and metal contents between different samples. The Correlation Analysis Tool of Microsoft Excel was used to analyze correlation coefficients between extracted P and metal contents.

RESULTS AND DISCUSSION

P Forms in Poultry Litter Stored in a Freezer

The sequential fractionation procedure effectively extracted P in the broiler litter sample (Table 2). The order of P distribution in the four fractions of frozen poultry litter was

TABLE 2. Metal and P Contents in Sequentially Extracted Fractions and the Residue of Poultry Litter and Biosolid Samples

Sample	Mg	Ca	Mn	Zn	Fe	Al	P
-----Water, mg kg^{-1} dry matter-----							
PL-RT	597 \pm 43a [†]	505 \pm 79a	10 \pm 6ab	31 \pm 3a	124 \pm 10a	19 \pm 5a	4,300 \pm 124a
PL-F	607 \pm 55a	501 \pm 54a	13 \pm 5a	35 \pm 14a	124 \pm 26a	16 \pm 4a	3,852 \pm 237b
BS	153 \pm 14b	516 \pm 45a	3 \pm 2b	1 \pm 1b	8 \pm 4b	4 \pm 1b	250 \pm 45c
-----NaHCO ₃ , mg kg^{-1} dry matter-----							
PL-RT	2,962 \pm 152a	2,931 \pm 49a	11 \pm 1a	7 \pm 2a	61 \pm 4a	21 \pm 1a	2,153 \pm 196a
PL-F	3,002 \pm 764a	2,958 \pm 343a	16 \pm 9ab	7 \pm 2ab	52 \pm 6a	10 \pm 7a	2,300 \pm 115ab
BS	684 \pm 8b	1,830 \pm 10b	20 \pm 0b	2 \pm 6b	1,194 \pm 1b	157 \pm 8b	2,879 \pm 109b
-----NaOH, mg kg^{-1} dry matter-----							
PL-RT	73 \pm 10a	1,540 \pm 104a	34 \pm 2a	28 \pm 2a	37 \pm 11a	775 \pm 42a	3,515 \pm 139a
PL-F	66 \pm 24a	1,611 \pm 157a	33 \pm 4a	26 \pm 7ab	48 \pm 2a	734 \pm 53a	3,734 \pm 391a
BS	21 \pm 1b	450 \pm 17b	57 \pm 2b	19 \pm 1b	220 \pm 20b	3,829 \pm 125b	9,023 \pm 222b
-----HCl, mg kg^{-1} dry matter-----							
PL-RT	1,940 \pm 272a	19,367 \pm 3,748a	318 \pm 33a	302 \pm 26a	459 \pm 36a	757 \pm 111a	6,644 \pm 886a
PL-F	2,000 \pm 349a	19,844 \pm 2,893a	352 \pm 11a	356 \pm 13ab	539 \pm 15a	724 \pm 79a	8,052 \pm 249a
BS	2,137 \pm 88a	30,246 \pm 236a	2,146 \pm 62b	541 \pm 12b	25,245 \pm 215b	7,088 \pm 218b	14,285 \pm 423b
-----Residue, mg kg^{-1} dry matter-----							
PL-RT	393 \pm 95a	1,492 \pm 7a	32 \pm 7a	30 \pm 9a	761 \pm 354a	72 \pm 24a	91 \pm 14a
PL-F	463 \pm 299a	1,442 \pm 14a	30 \pm 14ab	32 \pm 13ab	515 \pm 169a	40 \pm 10a	75 \pm 17a
BS	1,361 \pm 196b	752 \pm 5b	52 \pm 5b	51 \pm 8b	9,238 \pm 1,258b	1,753 \pm 71b	328 \pm 25b

PL-RT: poultry litter stored at room temperature; PL-F: poultry litter stored in a freezer.

[†]Mean \pm S.D. ($n = 3$). Different letters in a column of a fraction represent that these data are significantly different at $P = 0.05$.

BS: biosolids.

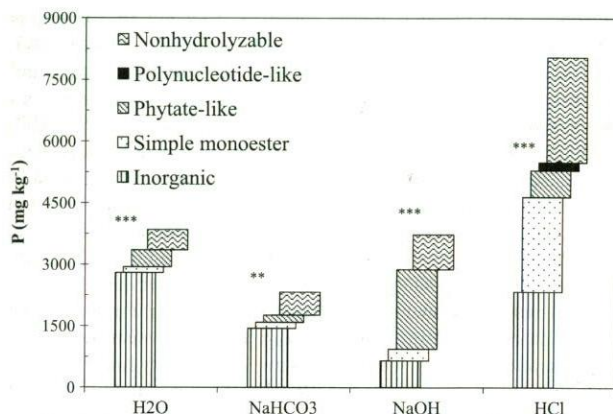


FIG. 1. Distribution of P forms in the four fractions of poultry litter stored in a freezer. ** And *** represent that P forms in the same fraction are significantly different at $P = 0.01$ and 0.001 , respectively.

HCl > H₂O > NaOH > NaHCO₃ with 8,052, 3,852, 3,734, and 2,330 mg kg⁻¹ of dry matter, respectively. The residues after all the sequential extractions contained only 75 mg P kg⁻¹ of dry matter. The observed greatest P amount presented in the HCl fraction is consistent with numerous previous reports (Codling, 2006; Dail et al., 2007; Dou et al., 2003; He et al., 2006b; 2008; Turner and Leytem, 2004). A different P distribution pattern in poultry litter and manure reported earlier (Sharpley and Moyer, 2000) was apparently caused by lack of organic P measurement in the HCl fractions. In the present study, organic P accounted for 59.7% of total P in the HCl extract (Fig. 1).

In all four fractions, more than half of the organic P was enzymatic hydrolyzable, mainly in the form of phytate or simple monoester (Fig. 1). The HCl fraction consisted of organic P mainly as simple monoester (2,312 mg P kg⁻¹) plus some phytate P (649 mg P kg⁻¹). This distribution of a great amount of organic P in the HCl fraction was consistent with other PL samples in previous reports by enzymatic hydrolysis (He et al., 2006b; 2008) or solution P-31 nuclear magnetic resonance spectroscopy (He et al., 2006b; 2008).

P Forms in Poultry Litter Stored at Room Temperature

Concentrations of P in the extracts of water, 0.5 M NaHCO₃ (pH 8.5), 0.1 M NaOH, 1 M HCl, and the residues of the broiler litter stored at room temperature for 15 months were 4,300, 2,153, 3,515, 6,940, and 91 mg kg⁻¹ of dry matter, respectively (Fig. 2). The distribution pattern was similar to that of the broiler litter stored in the freezer but with some differences in the quantities extracted. Total P calculated by the sum of the five fractions was 18,043 ± 1,156 mg kg⁻¹ for the broiler litter stored in the freezer, but 16,998 ± 1,268 mg kg⁻¹ for that stored at room temperature. However, the difference was not statistically significant at $P = 0.05$.

The amounts of P in the H₂O, NaHCO₃, NaOH, and HCl fractions of the sample stored at room temperature were 112%, 92%, 94%, and 86% of that stored in the freezer, respectively. Whereas P in the NaHCO₃ and NaOH fractions were similar in the samples stored differently, the substantial changes in the H₂O and HCl fractions must be caused by the different storage conditions. In fact, the distribution of P forms in the two fractions also changed. In the H₂O fraction of the sample stored at room temperature, the contents of inorganic P and phytate P decreased, whereas simple monoester P, polynucleotide-like P,

and nonhydrolyzable P increased compared with their counterparts in the freezer-stored sample. For the HCl fraction, the decrease in total P was mainly attributed to a decrease in simple monoester P, partially because of less phytate P. Similar to the H₂O fraction, however, nonhydrolyzable organic P increased. Assuming that P forms in the broiler litter stored frozen remained unchanged, we believe that storage at room temperature for 15 months converted some HCl-P to more labile P in the H₂O fraction. It seems that active conversion of organic P forms occurred during storage, leading to the transformation of phytate P to simple monoester P, which subsequently converted to nonhydrolyzable organic P. These observations are consistent with previous findings (He et al., 2003b), where enzymatically nonhydrolyzable P increased after 1 year of storage of swine manure at 4°C and cattle manure at 22°C, whereas soluble inorganic P and enzymatically hydrolyzable organic P remained relatively unchanged or decreased. Our data further demonstrate that manure P solubility may increase with storage, but the increase may not produce more bioavailable P (He et al., 2003b). On the other hand, Baxter et al. (2003) observed that storage of liquid pig slurry for 150 days reduced both soluble P_i and P_o, with the largest decreases occurring within 60 to 90 days.

P Forms in Biosolids Stored at Room Temperature

Total P in the biosolid sample was 26,935 mg kg⁻¹ of dry matter (Table 2), similar to an earlier report of 22,090 mg kg⁻¹ by Sui et al. (1999) but much higher than that by Ajiboye et al. (2004). In contrast to broiler litter, P in the four biosolid fractions increased with the order of extraction (Fig. 3). The same pattern was also observed for inorganic P, hydrolyzable organic P, and nonhydrolyzable organic P. Shepherd and Withers (2001) reported that more P was found in the HCl fraction of their digested sewage sludge samples than other fractions. Other studies (Ajiboye et al., 2004; Su et al., 2007; Sui et al., 1999) have shown that the greatest P is not in the HCl fraction, perhaps because of the failure to measure organic P in the HCl fraction. In our case, organic P accounted for 44.6% of the 14,285 mg kg⁻¹ P in the HCl fraction or 24% of total P in the biosolid sample. Without measured data, Huang et al. (2008) partially corrected the missing organic P portion in the HCl fraction of biosolids by acknowledging the possible presence of organic P in HCl fractions but included it in residual P. Clearly, inclusion of this portion of organic P in their own HCl fraction would lead to more accurate information and better understanding of biosolid P transformations once applied to soil.

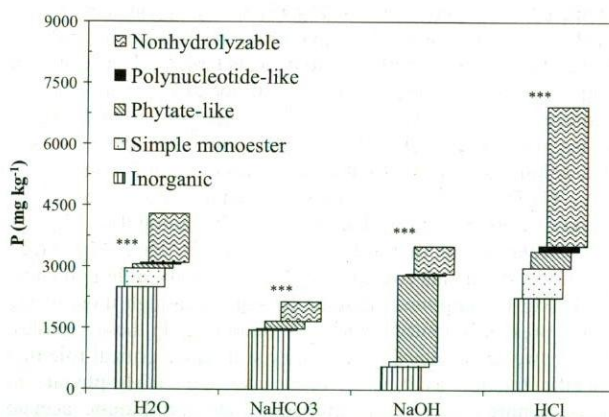


FIG. 2. Distribution of P forms in the four fractions of poultry litter stored at room temperature. ***P forms in the same fraction are significantly different at $P = 0.001$.

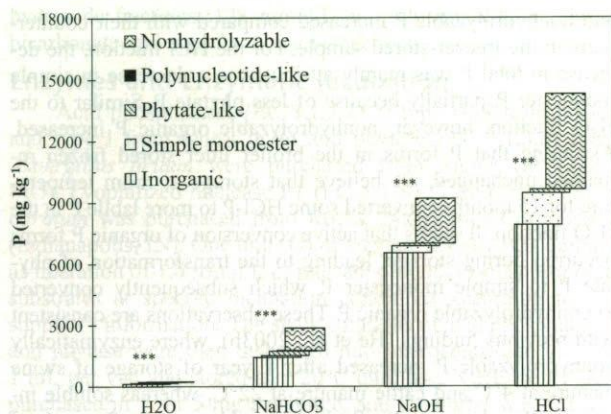


FIG. 3. Distribution of P forms in the four fractions of biosolids. ***P forms in the same fraction are significantly different at $P = 0.001$.

Unlike poultry litter, nonhydrolyzable P was the major form of organic P in biosolids. Phytate P (45 and 221 mg P kg⁻¹ in the H₂O and NaHCO₃ fractions, respectively) was greater than simple monoester P (35 and 88 mg P kg⁻¹ in the H₂O and NaHCO₃ fractions, respectively), but their absolute concentrations represented only a small portion of the total P. Phytate P concentration in the NaOH and HCl fractions differed, with 117 and 186 mg kg⁻¹, respectively. Simple monoester P was present at 342 and 1543 mg kg⁻¹ in the NaOH and HCl fractions, respectively. Little polynucleotide-like P was observed in all four fractions.

Metal Distribution in the Sequential Fractions of Poultry Litter and Biosolids

Almost all P (98.5%–98.8% of total P) in poultry litter and biosolid samples was recovered through the sequential extraction procedure (Table 2). Most metals were also extracted by the four-step fractionation, with recoveries greater than 90%. However, the recovery of Fe was only 47.2%, 59.3%, and 74.3% for poultry litter stored at room temperature (PL-RT), poultry litter stored in a freezer (PL-F), and biosolids stored at room temperature, respectively. This suggests that more than 50% of the Fe in these samples was not associated with P. Correlation analysis of all 12 sets of data (four fractions of each sample for three samples: PL-F, PL-RT, and BS) indicated that P was co-extracted with metals except Mg (Table 3). In other words, P in these PL and biosolid samples may be present in multiple metal-P compounds with different extractability. The results of correlation analysis are consistent with metal P species in dairy manure identified by solid-state P-31 nuclear magnetic resonance spectroscopy (He et al., 2009), which has demonstrated that soluble, moderate soluble, and stable metal-P compounds (i.e., Ca-P_i and P_o) and multiple metal-P (i.e., Al/Ca/Mg-phytate) were present in dairy manure. The amount of Mg was not correlated with that of P and other metals. Thus, Mg played less or no significant role in total P extractability, although some labile Mg-P compounds may be present. Although most of the P was present in multiple metal-P compounds in these samples, one or two major metal species may still play a critical role in a specific fraction as it is Al that makes Al/Ca/Mg-phytate in dairy manure insoluble in a moderate sodium dithionite–acetate buffer (pH 5.0) (He et al., 2009). With this assumption, we further analyzed the metal roles in P extractability in the PL and biosolid samples.

The total metal contents summed from the five fractions of biosolids (Table 2) were 4,356, 33,794, 2,278, 614, 35,905, and 12,831 mg kg⁻¹ of dry matter for Mg, Ca, Mn, Zn, Fe, and Al, respectively. In the meantime, the total metal contents measured with digestions were 3,205, 35,125, 2,315, 558, 27,902, and 13,568 mg kg⁻¹ of dry matter for the six metals, respectively. Thus, these two sets of metal content data were generally consistent and comparable, although they were not always the same. Maguire et al. (2001) reported that the contents of Ca, Fe, and Al of two biosolid samples were 8,000 and 29,000, 3,000 and 11,000, 4,000 and 9,000 mg kg⁻¹ of dry matter, respectively. Schroder et al. (2008) reported the Zn content of biosolids collected in 13 years from a similar waste treatment plant in a range from 709 to 1,340 mg kg⁻¹ of dry matter. These data indicated that the metal contents of our biosolid sample were at the same levels of other biosolids reported in literature. The biosolid sample contained greater amounts of metals (except for Mg) than the broiler litter, particularly for Fe and Al (Table 2). Although Ca and Mg may play more important roles than Fe and Al in P extractability in poultry litter (Cooperband and Good, 2002; He et al., 2006b), we might expect Fe and Al to play major roles in the solubility and partitioning of biosolid P in the four sequentially extracted fractions. Indeed, the distribution of extractable biosolid Al in the order of HCl > NaOH > NaHCO₃ > H₂O was similar to that observed for P distribution. On the other hand, Fe in the NaOH extract of biosolids was relatively low (220 mg kg⁻¹) compared with Al (3,829 mg kg⁻¹). Therefore, the major metal species of P in the NaOH fraction of biosolid P may be expected to be Al species. In addition, the less soluble metal ions (Mn and Zn) were also extracted in the same order of P partitioning: HCl > NaOH > NaHCO₃ > H₂O. We therefore conclude that the solubility of biosolid P was mainly controlled by these three metals (Al, Mn, and Zn), although the lower concentrations of Mn and Zn suggest that they may exert less control than Al. To our knowledge, no other biosolid P sequential fractionation reports have provided data on metal contents coextracted into the four fractions. Such data on P and metal ions coextracted by sequential fractionation could provide important information for understanding P-metal species in biosolids.

In some early studies (e.g., Cross and Schlesinger, 1995), P in the sequentially extracted NaOH and HCl fractions was frequently assigned to Fe/Al- and Ca-bound P. More recent publications have cautioned that such interpretation could be problematic because no direct experimental data have been reported on the chemical composition of these fractions

TABLE 3. Correlation Coefficients Between Extracted P and Metals

	P	Mg	Ca	Mn	Zn	Fe
Mg [†]	0.166ns					
Ca	0.789**	0.497ns				
Mn	0.826***	0.319ns	0.843***			
Zn	0.834***	0.408ns	0.990***	0.861***		
Fe	0.761**	0.262ns	0.732**	0.982***	0.752**	
Al	0.894***	0.063ns	0.627*	0.866***	0.665*	0.868***

[†]The correlation coefficients are calculated with 12 data points (mean of 12 extracts).

*, **, ***Statistical significance of the correlation coefficients at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

ns: no significance at $P > 0.05$.

(Sharpley and Moyer, 2000; He et al., 2003a). In the present work, the six metals we measured were distributed in all four fractions, indicating that various salts or mineral phases of these elements existed in these broiler litter and biosolid samples as previously proposed (He et al., 2003a; 2006b). Indeed, contents of the six metals were all greater in the HCl fractions of both samples than in the NaOH fractions, which suggested some Al/Fe-bound P could also be present in the HCl fractions. Another possibility is the existence of Ca/Al/Fe-P and/or other metal-P compounds in the samples that did not become soluble by a single extraction step. Consequently, it may not be appropriate to exclusively assign NaOH-extractable P to Al- and Fe-P, and HCl-extractable P to Ca-P (He et al., 2003a).

P Distribution in Granular Products of Poultry Litter and Biosolids

Poultry litter alone (PLU and PLUDCD) or poultry litter and biosolid together (PLUB and PLUBDCD) were used to make the granular fertilizer products (Table 1). All P forms were impacted by granulation process, but the relevant abundance of the different P forms in most fractions was similar to the corresponding fractions of poultry litter or litter/biosolid combination (data not shown), indicating cotransformation of both

inorganic and organic P between different fractions in these granulated products. Thus, we presented and analyzed the total P changes impacted by granulation (Table 4). Phosphorus distribution in these granulated products was not a simple combination of P contributed from each fraction of two materials as the P levels of most fractions of these products were significantly different from the calculated values (Table 4). Dicyandiamide (DCD) did not play a major role in P solubility in these products because P concentrations in most fractions of granulated products with or without DCD (i.e., PLU vs. PLUDCD or PLUB vs. PLUBDCD) were similar (Table 4). The effects of urea could not be evaluated because all granulated products contained urea in a range from 250 to 204 kg t⁻¹.

Compared with the assumed values, water-extracted P significantly increased in five of six poultry litter-only granulated products. The increase apparently came from P in moderate NaHCO₃- and/or NaOH-extractable P in poultry litter as P in the two fractions decreased compared with their corresponding assumed values (Table 4). Interestingly, stable P in the HCl fractions in poultry litter-only granulated products was higher than their assumed values. The most recalcitrant P that was in the residues after the sequential extraction also increased after granulation. Thus, a part of the moderately stable P in poultry

TABLE 4. P Distribution in Granular Products of Poultry Litter and Biosolids

Product	Assumed [†]	LS	UF	Water
-----H ₂ O, mg kg ⁻¹ dry matter-----				
PLU	2,889 ± 178a [‡]	3,553 ± 182b	3,558 ± 335b	3,425 ± 241b
PLUDCD	2,916 ± 180a	3,527 ± 100b	3,344 ± 131b	2,892 ± 821ab
PLUB	1,804 ± 95a	2,004 ± 44b	1,833 ± 173ab	2,062 ± 204ab
PLUBDCD	1,836 ± 97a	1,794 ± 243a	1,833 ± 106a	1,995 ± 151a
-----NaHCO ₃ , mg kg ⁻¹ dry matter-----				
PLU	1,243 ± 216a	1,065 ± 23a	925 ± 82a	1,006 ± 53a
PLUDCD	1,255 ± 218a	1,096 ± 67a	915 ± 67ab	835 ± 216ab
PLUB	1,641 ± 131a	1,810 ± 52ab	1,546 ± 126a	1,871 ± 39b
PLUBDCD	1,672 ± 134a	1,688 ± 156ab	1,630 ± 126a	1,942 ± 72b
-----NaOH, mg kg ⁻¹ dry matter-----				
PLU	2,800 ± 293a	1,490 ± 191b	1,895 ± 36c	1,951 ± 160c
PLUDCD	2,826 ± 296a	1,269 ± 149b	1,970 ± 74c	1,768 ± 233c
PLUB	4,488 ± 160a	4,422 ± 183a	4,196 ± 208a	4,410 ± 128a
PLUBDCD	4,572 ± 163a	4,476 ± 120a	4,057 ± 47b	4,725 ± 246a
-----HCl, mg kg ⁻¹ dry matter-----				
PLU	6,039 ± 132a	7,278 ± 364b	7,326 ± 949ab	7,990 ± 712b
PLUDCD	6,095 ± 133a	8,477 ± 367b	7,843 ± 363bc	7,406 ± 2348c
PLUB	8,064 ± 194a	5,526 ± 252b	5,973 ± 227b	5,746 ± 265b
PLUBDCD	8,214 ± 198a	5,804 ± 129b	5,357 ± 174c	5,819 ± 226b
-----Residual, mg kg ⁻¹ dry matter-----				
PLU	56 ± 12a	957 ± 395b [§]	ND	ND
PLUDCD	57 ± 13a	586 ± 261b	ND	ND
PLUB	136 ± 8a	1,773 ± 500b	ND	ND
PLUBDCD	138 ± 8a	1,939 ± 207b	ND	ND

[†]Calculated from the percentages of poultry litter and biosolids.

[‡]Mean of triplicates. Different letters in the same row represent that these data are significantly different at $P = 0.05$.

[§]Difference between total product P and sum of P in the four fractions. Total P was 14,343, 14,458, 15,204, and 15,702 mg kg⁻¹ dry matter, respectively, for the four products in order. Total P was analyzed by digesting the samples and then inductively coupled plasma optical emission spectroscopy.

PLU: poultry litter + urea; PLUDCD: poultry litter + urea + DCD; PLUB poultry litter + urea + biosolids; PLUBDCD: poultry litter + urea + biosolids + DCD.

ND: not determined; LS: lignosulfonate; UF: urea formaldehyde resin + catalyst.

litter had been transferred to either more labile water-extractable P or more recalcitrant HCl-extractable and residual P in the granulated products. As little difference in P concentrations were observed in the same fractions with the three binding agents (lignosulfonate, urea formaldehyde resin with catalyst, and water), we attributed the changes in P distribution in these granular products to the drying (heating) effect during the granulation. Previously, Dail et al. (2007) observed a similar bilateral trend of poultry manure P change during drying.

We also observed the differences in the assumed values and P concentrations in fractions of poultry litter-and-biosolid-together granulated products (i.e., PLUB and PLUBDCD). However, some differences were not statistically significant (Table 4). Furthermore, the trend of impacts by granulation differed from that of poultry litter-only granulated products. In these poultry litter-and-biosolid-together granulated products, P concentrations in the H₂O, NaHCO₃, and NaOH fractions did not remarkably deviate from the assumed values. In contrast, the P concentrations in the HCl fractions decreased in a range from 2100 to 2900 mg kg⁻¹ dry matter compared with the assumed values (Table 4). Most of the disappeared HCl-extractable P seemed to occur in the residual fractions, as shown by the increase in residual P in the two lignosulfonate-bound products. Apparently, the high concentrations of divalent and trivalent metals from the biosolids (Table 2) made the difference in impacts of granulation on P distribution between poultry litter-only products and poultry litter-and-biosolid-together granulated products.

Previously, Toor et al. (2007a) and Haggard et al. (2005) observed that granulation or pelletizing of poultry litter led to an increase in water-soluble P of poultry litter, raising concern that land application of these processed litter products may increase P loss to waters. Toor et al. (2007a) further demonstrated that the level of water-soluble P was similar for all litters (raw, ground, heated, and granulated) when measured at higher extraction ratios (>1:200, g:mL). This observation could be explained by the fact that part of moderate NaHCO₃-extractable P became water soluble P at higher extraction ratios. Our data in this work demonstrated that granulation of poultry litter with biosolids could relieve the concern. On the other hand, granulation had a part of NaOH-extractable P transformed to more stable HCl and recalcitrant residual P in granulated litter, and even HCl-extractable P to residual P in the granulated litter/biosolid mixing products. We assume that the heating is the major cause of the transformation as such similar changes have been observed in drying poultry manure (Dail et al., 2007). Under extreme conditions of ashing, most (80% or so) P in poultry litter is transferred into the HCl fraction (Codling, 2006). Either phase change or decomposition of organic matter caused by heating and drying might have led to the formation of tightly bound metal P compounds and/or minerals, although further in-depth studies are needed to elucidate the mechanisms of these changes.

CONCLUSIONS

Both broiler litter and biosolids contained significant amounts of P. Sequential fractionation by H₂O, 0.5 M NaHCO₃ (pH 8.5), 0.1 M NaOH, and 1 M HCl effectively extracted P from the two types of organic samples, with 99.5% recovery. The order of P distribution in the four fractions was HCl > H₂O > NaOH > NaHCO₃ for broiler litter, but HCl > NaOH > NaHCO₃ > H₂O for biosolids. Compared with P levels of the sample stored in a freezer, storage of the broiler sample at room temperature had transformed a part of its phytate P in the HCl fraction to simple monoester, which was further converted

to nonhydrolyzable organic P. In the H₂O fraction of the sample stored at room temperature, the contents of inorganic P and phytate P decreased, accompanied by increases in simple monoester P, polynucleotide-like P, and nonhydrolyzable P. The combined result was that broiler litter storage at room temperature shifted some HCl-extractable P to more labile P in the H₂O fraction.

Granulation impacted P distribution patterns in granulated litter or litter/biosolid products. Granulation transformed moderate amounts of NaHCO₃- and NaOH-extractable P to labile P (water soluble) and stable P (HCl-extractable and residual) in poultry litter-only granulated products but did not remarkably change the distribution of H₂O-, NaHCO₃-, and NaOH-extractable P in the granulated products containing both poultry litter and biosolids. This observation suggests that the high concentration of metals (especially Al) in biosolids reduced the impact of granulation on water-soluble P increase in these granulated products. Therefore, the litter/biosolids mixing granulated products could serve better as slow-release fertilizer by reducing water-soluble P levels in these products, thus alleviating the concerns of P runoff to waters in soils amended with poultry litters.

REFERENCES

- Ajiboye, B., O. O. Akinremi, and G. J. Racz. 2004. Laboratory characterization of phosphorus in fresh and oven-dried organic amendments. *J. Environ. Qual.* 33:1062–1069.
- Alleoni, L. R. F., S. R. Brinton, and G. A. O'Connor. 2008. Runoff and leachate losses of phosphorus in a sandy Spodosol amended with biosolids. *J. Environ. Qual.* 37:259–265.
- Baxter, C. A., B. C. Joern, D. Ragland, J. S. Sands, and O. Adeola. 2003. Phytase, high-available-phosphorus corn, and storage effects on phosphorus levels in pig excreta. *J. Environ. Qual.* 32:1481–1489.
- Codling, E. E. 2006. Laboratory characterization of extractable phosphorus in poultry litter and poultry litter ash. *Soil Sci.* 171:858–864.
- Cooperband, L. R., and L. W. Good. 2002. Biogenic phosphate minerals in manure: implications for phosphorus loss to surface waters. *Environ. Sci. Technol.* 36:5075–5082.
- Cross, A. F., and W. H. Schlesinger. 1995. A literature review and evaluation of the Hedley fractionation: applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64:197–214.
- Dail, H. W., Z. He, M. S. Erich, and C. W. Honeycutt. 2007. Effect of drying on phosphorus distribution in poultry manure. *Commun. Soil Sci. Plant Anal.* 38:1879–1895.
- Dou, Z., G. Y. Zhang, W. L. Stout, J. D. Toth, and J. D. Ferguson. 2003. Efficacy of alum and coal combustion by-products in stabilizing manure phosphorus. *J. Environ. Qual.* 32:1490–1497.
- Gerritse, R. G., and I. Zúguez. 1977. The phosphorus cycle in pig slurry measured from 32PO₄ distribution rates. *J. Agric. Sci. (Cambridge)* 88:101–109.
- Haggard, B. E., P. A. Vadas, D. R. Smith, P. B. DeLaune, and P. A. Moore Jr. 2005. Effects of poultry litter to water ratios on water-extractable phosphorus and its relation with runoff phosphorus concentrations. *Biosys. Eng.* 92:409–417.
- He, Z., and C. W. Honeycutt. 2005. A modified molybdate blue method for orthophosphate determination suitable for investigating enzymatic hydrolysis of organic phosphates. *Commun. Soil Sci. Plant Anal.* 36:1373–1383.
- He, Z., C. W. Honeycutt, and T. S. Griffin. 2003a. Comparative investigation of sequentially extracted P fractions in a sandy loam soil and a swine manure. *Commun. Soil Sci. Plant Anal.* 34:1729–1742.
- He, Z., C. W. Honeycutt, and T. S. Griffin. 2003b. Enzymatic hydrolysis of organic phosphorus in extracts and resuspensions of swine manure and cattle manure. *Biol. Fertil. Soils.* 38:78–83.

- He, Z., T. S. Griffin, and C. W. Honeycutt. 2004a. Enzymatic hydrolysis of organic phosphorus in swine manure and soil. *J. Environ. Qual.* 33:367–372.
- He, Z., T. S. Griffin, and C. W. Honeycutt. 2004b. Phosphorus distribution in dairy manures. *J. Environ. Qual.* 33:1528–1534.
- He, Z., T. S. Griffin, and C. W. Honeycutt. 2006a. Soil phosphorus dynamics in response to dairy manure and inorganic fertilizer applications. *Soil Sci.* 171:598–609.
- He, Z., Z. N. Senwo, R. N. Mankolo, and C. W. Honeycutt. 2006b. Phosphorus fractions in poultry litter characterized by sequential fractionation coupled with phosphatase hydrolysis. *J. Food Agric. Environ.* 4:304–312.
- He, Z., C. W. Honeycutt, B. J. Cade-Menun, Z. N. Senwo, and I. A. Tazisong. 2008. Phosphorus in poultry litter and soil: enzymatic and nuclear magnetic resonance characterization. *Soil Sci. Soc. Am. J.* 72:1425–1433.
- He, Z., A. Fortuna, Z. N. Senwo, I. A. Tazisong, C. W. Honeycutt, and T. S. Griffin. 2006c. Hydrochloric fractions in Hedley fractionation may contain inorganic and organic phosphates. *Soil Sci. Soc. Am. J.* 70:893–899.
- He, Z., C. W. Honeycutt, T. S. Griffin, B. J. Cade-Menun, P. J. Pellechia, and Z. Dou. 2009. Phosphorus forms in conventional and organic dairy manure identified by solution and solid state P-31 NMR spectroscopy. *J. Environ. Qual.* 38:1909–1918.
- Hedley, M. J., R. E. White, and P. H. Nye. 1982. Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings: III. Changes in L value, soil phosphate fractions and phosphatase activity. *New Phytol.* 91:45–56.
- Huang, X.-L., Y. Chen, and M. Shenker. 2008. Chemical fractionation of phosphorus in stabilized biosolids. *J. Environ. Qual.* 37:1949–1958.
- Ippolito, J. A., K. A. Barbarick, and K. L. Norvell. 2007. Biosolids impact soil phosphorus accountability, fractionation, and potential environmental risk. *J. Environ. Qual.* 36:764–772.
- Maguire, R. O., J. T. Sims, S. K. Dentel, F. J. Coale, and J. T. Mah. 2001. Relationships between biosolids treatment process and soil phosphorus availability. *J. Environ. Qual.* 30:1023–1033.
- Paudel, K. P., and C. S. McIntosh. 2005. Country report: Broiler industry and broiler litter-related problems in the southeastern United States. *Waste Management* 25:1083–1088.
- Schroder, J. L., H. Zhang, D. Zhou, N. T. Basta, W. R. Raun, M. E. Payton, and A. Zazulak. 2008. The effect of long-term annual application of biosolids on soil properties, phosphorus, and metals. *Soil Sci. Soc. Am. J.* 72:73–82.
- Sharpley, A. N., and B. Moyer. 2000. Phosphorus forms in manure and compost and their release during simulated rainfall. *J. Environ. Qual.* 29:1462–1469.
- Shepherd, M. A., and P. J. Withers. 2001. Phosphorus leaching from digested sewage sludge applied to sandy soils. *J. Agric. Sci.* 68:2048–2057.
- Su, J., H. Wang, M. O. Kimberley, K. Beecroft, G. N. Magesan, and C. Hu. 2007. Fractionation and mobility of phosphorus in a sandy forest soil amended with biosolids. *Env. Sci. Pollut. Res.* 14:529–535.
- Sui, Y., M. L. Thompson, and C. Shang. 1999. Fractionation of phosphorus in a Mollisol with biosolids. *Soil Sci. Soc. Am. J.* 63:1174–1180.
- Singh, R. P., and M. Agrawal. 2008. Potential benefits and risks of land application of sewage sludge. *Waste Management*. 28:347–358.
- Toor, G. S., and B. E. Haggard. 2009. Phosphorus and trace metal dynamics in soils amended with poultry litter and granulates. *Soil Use Manag.* 25:409–418.
- Toor, G. S., B. E. Haggard, M. S. Reiter, T. C. Daniel, and A. M. Donoghue. 2007a. Phosphorus solubility in poultry litters and granulates: Influence of litter treatments and extraction ratios. *Transactions. ASAE* 50:533–542.
- Toor, G. S., B. E. Haggard, and A. M. Donoghue. 2007b. Water extractable trace elements in poultry litters and granulated products. *J. Appl. Poultry Res.* 16:351–360.
- Toor, G. S., B. E. Haggard, S. M. Williamson, and A. M. Donoghue. 2007c. Less environmental effects from granulated litter. *World Poultry*. 23:26–27.
- Toth, J. D., Z. Dou, J. D. Ferguson, D. T. Galligan, and C. F. Ramberg Jr. 2006. Nitrogen- vs. phosphorus-based dairy manure applications to field crops: nitrate and phosphorus leaching and soil phosphorus accumulation. *J. Environ. Qual.* 35:2303–2312.
- Turner, B. L., and A. B. Leytem. 2004. Phosphorus compounds in sequential extracts of animal manure: chemical speciation and a novel fractionation procedure. *Environ. Sci. Technol.* 38:6101–6108.
- Vadas, P. A., W. J. Gburek, A. N. Sharpley, P. J. A. Kleinman, P. A. Moore Jr., M. L. Cabrera, and R. D. Harmel. 2007. A model for phosphorus transformation and runoff loss for surface-applied manure. *J. Environ. Qual.* 36:324–332.